chment 1.
E SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date October 01, 1990
24.7.91

cipal Investigator Dr.Muzahed Uddin Ahmed
ication No. PCC/009/90
e of Study Antigenic characterization human rotavirus serotypes by ELISA ~~and RNA electropherotyping in Mymensingh~~

Trainee Investigator (if any) None
Supporting Agency (if Non-ICDDR,B)

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

le the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects Yes (No)
(c) Minors or persons under guardianship (Yes) No

Does the study involve:
(a) Physical risks to the subjects Yes (No)
(b) Social Risks Yes (No)
(c) Psychological risks to subjects Yes (No)
(d) Discomfort to subjects Yes (No)
(e) Invasion of privacy Yes (No)
(f) Disclosure of information damaging to subject or others Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) (Yes) No
(b) Use of fetal tissue or abortus Yes (No)
(c) Use of organs or body fluids Yes (No)

Are subjects clearly informed about:
(a) Nature and purposes of study (Yes) No
(b) Procedures to be followed including alternatives used Yes No
(c) Physical risks Yes (No)
(d) Sensitive questions Yes (No)
(e) Benefits to be derived (Yes) No
(f) Right to refuse to participate or to withdraw from study (Yes) No
(g) Confidential handling of data (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes (No)

5. Will signed consent form be required:
   (a) From subjects (Yes) No
   (b) From parent or guardian (if subjects are minors) (Yes) No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ___ Protocol (Required)
   ✓ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ✓ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

ree to obtain approval of the Ethical Review Committee for any changes ving the rights and welfare of subjects before making such change.

Muahmed

Principal Investigator

Trainee

PEC/009/90
24/7/91

DEPARTMENT OF HYGIENE AND EPIDEMIOLOGY
SAPPORO MEDICAL COLLEGE
SOUTH-1, WEST-17, CHUO-KU
SAPPORO, 060 JAPAN

July 13, 1990

Dr. Muzahed Uddin Ahmed
Associate Professor
Department of Medicine
Faculty of Veterinary Science
Bangladesh Agricultural University
Mymensingh 2202, BANGLADESH

Dear Dr. Ahmed,

Thank you for your letter of June 24. As regards your proposal for the collaborative study on antigenic characterization of human rotavirus prevailing in Mymensingh, Bangladesh, we are quite prepared to accept your proposal. In that case we offer serotype-specific monoclonal antibodies of required amount with the conditions described below.

1. The results should be published as a collaborative study.
2. All samples should be divided into two portions; one is used for ELISA serotyping in your or other appropriate laboratory and the other is reserved at $< -20°C$ for later reconfirmation experiment.
3. The data obtained should be sent to us beforehand, and be published only after the consent of both sides.
4. When unusual results are obtained, those samples stored at $< -20°C$ should be sent to Department of Hygiene and Epidemiology for reconfirmation.

You may, of cause, send the virus-positive specimens to our laboratory by air mail, if your laboratory is not yet ready to conduct serotyping of stool specimens.

Yours sincerely,

Shozo Urasawa

Shozo Urasawa, M.D., Professor
Department of Hygiene
and Epidemiology
Sapporo Medical College
South-1, West-17, Chuo-ku
Sapporo, 060 JAPAN

SECTION -I : RESEARCH PROTOCOL

1. Title : Antigenic characterization of human rotavirus Serotypes by ELISA and RNA electropherotyping in Mymensingh.

2. Principal Investigator : Dr. Muzahed Uddin Ahmed
Muahmed 18/9/90
Associate Professor,
Department of Medicine,
Bangladesh Agricultural University
Mymensingh.

3. Co-investigators : 1. Dr. M.A. Hamid Shaikh,
Associate Professor
Department of Pediatrics,
Mymensingh Medical College
Mymensingh.
Associate Professor And Head of the Department of Pediatrics Mymensingh Medical College
2. Dr. Nigar Shahid,
Associate Scientist, ICDDR, B, Dhaka.
3. Dr. Shozo Urasawa,
Professor, Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, 060 Japan.

4. Consultant : Dr. A.I.M. Mafakhkharul Islam,
Professor of Community Medicine & Principal Mymensingh Medical College
Principal Mymensingh Medical College
Mymensingh.

5. Starting date : As and when protocol is approved

6. Completion date : One year from the date of commencement

7. Total direct cost : 3,50,000.00

8. Recommendations :

(a) National institution

Signature of Director
BAURES, BAU, Mymensingh.

Date 22.9.90

Director
BAU Research System
Bangladesh Agricultural University
Mymensingh

(b) ICDDR, B.

Signature of Associate Director, LSD.

Date _____

Abstract Summary :

A recent study on diarrheal hospital patients in Mymensingh indicated that the 30% of the patients were associated with group A rotaviruses. Subsequently group A positive specimens were further characterized by ELISA using subgroup and serotype specific monoclonal antibodies (Mabs). We observed that subgroup I was more prevalent than subgroup II in both the hospitals in first phase of the study although some specimens reacted with both subgroup I- and subgroup II- specific Mabs. On the contrary, subgroup II was predominant than subgroup I in the second phase of the study. A large portion of the stool specimens collected in the second phase could not be characterized either by subgroup I-or subgroup II- specific Mabs, although they reacted very strongly with group A specific Mabs.

The serotype specificities were also analysed by using serotype specific neutralizing Mabs. While the frequency of serotype 2 was highest in both the hospitals which lends contrast to our previous findings in which serotype 1 was most prevalent. The predominant serotype varied from first phase of study(January to June 1988) to second phase (July 1988 to December 1989). Within a single year in Mymensingh area rotavirus serotypes were not equally distributed. These differences in the distribution of group A rotavirus serotypes have broad implications for the design and interpretation of vaccine programmes. We have identified 16 different electropherotypes based on the differences in the mobility between two different RNA segments. A large number of the specimens collected during nation-wide flood in 1988 showed mixed electropherotypes.

The results mentioned above merit an extension of the study to know rotavirus serotype distribution in Mymensingh area. Keeping these facts in view we propose:

(1) To extend the study of antigenic characterization of HRV using subgroup and serotype specific monoclonal antibodies in stool specimens from diarrheic patients in Mymensingh.

(2) To extend the study of RNA electropherotyping of HRV in Mymensingh.

(3) To determine the presence of atypical rotaviruses and their association with diarrhea.

SECTION - II : RESEARCH PLAN

INTRODUCTION

1. Objective:

1.1 Antigenic characterization of human rotaviruses by ELISA using subgroup and serotype specific monoclonal antibodies in stool specimens of diarrheic patients in Mymensingh.

1.2 Molecular epidemiology of human rotaviruses prevaling in Mymensingh by RNA electropherotyping of double stranded RNA (ds RNA) genome.

1.3 Determination of atypical rotaviruses with association of diarrhoea in rural Bangladesh.

1.4 To determine whether animal strains of rotaviruses are a cause of infection in man.

Background information:

Group A rotaviruses are the single most important cause of severe infantile diarrheal disease in both developed and less developed countries(1,2). In a variety of studies from developed and less developed countries, it has been shown that rotavirus accounts for approximately 30-40% of all the hospitalization for severe dehydrating diarrhea in children under the age of five (2,3,4,5,6,7). In less developed countries where malnutrition is common among infants and young children, severe dehydration following rotavirus diarrhea certainly leads to high rate of mortality (1,3,8). In Bangladesh, a comprehensive study of the etiology of moderate to severe diarrheal illness has indicated the presence of rotaviruses among children(3,9). Subsequent studies also revealed the higher prevalence of rotaviruses in the diarrheal episodes, suggesting the potential of rotavirus to cause life threatening diarrhea in young children(2,5,6,9).

Our recent survey on diarrheal patients in two hospitals in Mymensingh from January 1988 to December 1989 indicated that 290(30%) of 983 patients were associated with group A rotaviruses (10). Subsequently group A positive stool specimens were further characterised by ELISA using subgroup and serotype specific Mabs(11). We observed the predominance of subgroup II than subgroup I in both th the hospitals (10,11,12). These findings were in contrast to our previous observation where subgroup I was more prevalent. A large portion of specimens could not be determined either by subgroup I

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or subgroup II specific monoclonal antibodies, although they reacted very strongly with group A specific Mab. These result suggest a possible existence of a third subgroup of HRV as reported recently(13). The serotype specificities were analysed by using ser serotype specific neutralizing Mabs. The frequency of serotype 2 was highest in both the hospitals which lends contrast to our previous findings in which serotype 1 was most prevalent. The RNA electropherotyping showed a variety of RNA profiles based on the differences in the mobility between the two different RNA segments. As a result 16 different electropherotypes were identified. Interestingly a large number of the specimens collected during nationwide flood in 1988 showed mixed electropherotypes. In Bangladesh very little information on the subgroups and serotypes distribution is available. As the prevalence of HRV subgroups and serotype vary from year to year, we would like to extend our study to know the differences in the distribution of group A rotavirus subgroups and serotypes in stool specimens of diarrheic patients in these hospitals in Mymensingh. Human rotavirus has two major antigenic specificities i.e. subgroup (14,15,16), and serotype (17,18,19). The presence of six antigenically distinct serotypes have been recognized by neutralization test(18,19). Epidemiological data on each serotype is however insufficient mainly because of a simple and rapid method for serotyping HRV isolates has not been developed. The epidemiological features of infection of each HRV serotypes have also not been throughly studied for the same reason. Recently enzymelinked immunosorbent assay (ELISA) for directly serotyping HRVs in stool specimens using serotype 1,2,3 and 4 specific neutral neutralizing monoclonal antibodies has been developed(20).

By using this method the present study will be undertaken to determine subgroup and serotype specific antigens of individual HRV serotypes prevalent in Mymensingh.

Further more, there is a correlation between subgroup and serotype specificities; subgroup I strains are serotype 2 and subgroup II strains are serotype 1,3 and 4. Subgroup specificity is associated with VP6 of the inner capsid while serotype specificity is associated with an outer capsid protein VP7. Further, recently a novel strain with subgroup II and serotype 3 has been

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described. In experimental conditions, reassortment between two different strains can readily occur. By examining subgroup speci specificity of the strains in addition to serotype specificity we can expect to detect such unusal strains in nature as a possible result of reassortment. In recent years it has become clear that antigenically and genetically distinct (atypical) rotaviruses also infect the intestinal tract of man and animals(21). These rotaviruses do not share common antigens with group A rotaviruses and hence are not detected with the commonly employed rotavirus detection immunoassay (21). In some animal species these strains now appear to be the most frequently encountered rotaviruses(22). Because these atypical rotaviruses have a characteristic electropherotype of their dsRNA genome, they can be recognized by screening fecal specimen on PAGE and staining with silver stain(21). In limited studies the atypical strains have been infrequently encountered except in China where they have been the cause of very large epidemics of diarrhea(23). The origin of the atypical human rotaviruses is not clear but they may be derived from animal strains. There is no information concerning the prevalence of these strains in rural Bangladesh. We intend to determine if the atypical strains play a role in infantile diarrhea in this setting. We will also conduct subgroup testing on all rotavirus positive specimen (14,24). We will specifically look to identify subgroup I strains with long electropherotypes. Such strains, on a statistical basis, have a high liklihood for being of animal origin. If such isolates are identified, they will be studied further by cultivation and serotype analysis using antisera to animal rotavirus, RNA-RNA hybri hybridization and RNA sequence analysis (21,25,26). Such analysis will enable us to determine if the putative animal strain is indeed more related to other animal strains than to human rotavirus. The RNA-RNA hybridization and RNA sequence studies will be done in the laboratory of Dr. Shozo Urasawa, Sapporo Medical College, Japan.

General Aim:

Human rotavirus (HRV) has been recognized as the major etiologic agent a causing acute gastroenteritis in infants and young children; severe dehydration following rotavirus diarrhea leads to high rate of mortality. Development of an effective rotavirus vaccine

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therefore is the urgent need to control the rotavirus diarrhea. The presence of at least six serotype of HRV have hampared the development of broadly protective HRV vaccine. Indeed recent vaccine trial studies using rhesus rotavirus (RRV) with serotype 3 specificity showed the failure of protection against rotavirus illness due to heterotypic strains (27). Thus extensive epidemiological studies on HRV serotypes in less developed countries are required for the development and evaluation of the vaccine. Therefore we propose to characterize HRV from stool specimens of hospitalized diarrheic patients in Mymensingh.

Specific aims :

1. To extend the study of antigenic characterization of HRV using subgroup and serotype specific monoclonal antibodies in stool specimens from diarrheic patients in Mymensingh.
2. To extend the study of RNA electropherotyping of human rotaviruses in Mymensingh by RNA electropherotyping of double stranded RNA(ds RNA) genomic segments.
3. To determine the presence of atypical rotaviruses, whether atypical rotaviruses are important pathogen in rural Bangladesh, and to determine whether animal strains of rotaviruses are a cause of infection in man.

Ethical implications:

Stool samples or rectal swabs sample will be required. A written consent will be obtained from gaurdians of minors and patients themselves in case of adults.

Methods of procedure:

Study area: The proposed study will be carried out in Mymensingh with heavy rainfall during the months of May, June, July, August, September and October. The annual mean temperature ranges from 19 to 28°C and the relative humidity ranges from 76% to 88% throughout the year.

Patients:

Patients will be enrolled for one calendar year. Stool samples will be collected from infants, young children, adults and calves with watery diarrhea from hospitals and dairy farm of BAU, Mymensingh. It is expected that ten subjects will be enrolled/week for a calander year. Samples will be tested simultaneously, entered monthly and data analysis will be performed every 6 months.

Grouping, subgrouping and serotyping of HRV by ELISA with monoclonal antibodies:

The procedure of ELISA for grouping, subgrouping and serotyping of HRV is essentially the same. As capture antibodies, eight monoclonal antibodies will be used in the test. The antibody YO-156, reacting with the group A common antigenic epitope in the inner capsid protein VP6, and YO-202, reacting with the cross-reactive neutralization epitope in the outer capsid protein VP4 will be employed to confirm group A rotavirus antigen in stool specimens; antibodies S2-37 and YO-5 each reacting specifically with subgroup 1 and subgroup II specific epitopes in VP6, will be employed for subgrouping of group A rotaviruses, antibodies KU-4, S2-2G10, YO-1E2 and S2-2G7, each recognizing specifically serotype 1 through 4 specific neutralization epitopes, in the outer capsid protein VP7 will be used for serotyping viruses. In this study ELISA will be carried out with microtitre plate using the respective monoclonal antibody as capture antibody(Ascitic fluid) and rabbit anti-serum as a detector antibody. The group and subgroup specific monoclonal antibodies will be used in characterization of animal strains(19).

Briefly, microtitre plates will be coated with 1:10,000 dilution of ascitic fluid will be incubated overnight at 4°C. After washing the wells 1% bovine serum albumin will be added and incubated overnight at 4°C, subsequently washed mixture of 10% stool suspension and skim milk will be allowed to react in the wells for overnight at 4°C. After washing 50ul of rabbit anti HRV serum 1:10,000 dilution will be added to each well and incubated for 1 hour at 37°C. After washing 50 ul of peroxidase conjugated goat antirabbit immunoglobulin will be added and incubated for 1 hour at 37°C and washed. The reaction with the substrate OPD will be allo allowed to develop for 30 min. at room temperature and then stabalized by addition of 25 ul of 3N sulfuric acid. The optical density will be measured at 492 nm with a ELISA reader.

RNA electropherotyping of rotaviruses:

A 500 ul of 10% stool suspension will be clarified after low speed centrifugation. The supernatent will be mixed with 10mM Tris hydrochloride buffer. The virus will be disrupted with sodium dodecyl sulphate, 2-mercapatoethanol and EDTA. The genomic double

contd.

stranded RNA will be extracted with phenol chloroform, precipitated with ethanol and then analyzed by polyacrylamide gel electrophoresis on 10% slab gels. After electrophoresis at 40mAmp for 6 hours the double stranded RNA bands will be visualized by the silver staining method. Co-electrophoresis of stool specimens will also be performed to classify different electropherotypes that appear to show differences in migration pattern.

Significance:

The proposed study will help to examine the relative frequency of rotavirus subgroup I and II in stool specimens of hospitalized patients in relation with specific clinical symptoms, seasonal variation and age of infection.

The presence of six antigenically distinct serotypes have been reported in group A human rotaviruses. The epidemiological features of infection for each HRV serotypes have not been throughly studied in Bangladesh. The present study will be performed to know the prevalence of individual HRV Serotypes and to determine the antigenic specificities of group A rotaviruses in stool specimens. Recent vaccine trial studies suggest that thepprotection phenomenon against rotavirus illness is serotype specific. Also differences in the distribution of group A rotavirus serotypes have broad implication for the design and interpretation of vaccine programe. Thus extensive epidemiological studies on HRV serotypes in Bangladesh is essential before and after introduction of HRV vaccine. Aitypical rotaviruses are infrequently encountered in man and not clearly associated with diarrheal disease. The aim of this study is also to determine the incidence of atypical rotavirus infection in Mymensingh.

Facilities required:

Laboratory space and some equipment like, chilled centrifuge, Gel apparatus for Electrophoresis, water still plant, PH meter, deep freeze, refrigerator, micropipetts and other basic equipment are available at the Department of Medicine BAU. Additional equipment support will be needed to undertake this study which has been included in the budget.

References:

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SECTION III : BUDGET

1. Personal Service

Name	Time required	Honorarium/ Salary
Dr.Muzahed Uddin Ahmed Principal Investigator	50%	Tk.3,000X12=36,000.00
Dr. Nigar Sahid Co-investigator	5%	-
Dr.M.A.Hamid Shaikh Co-investigator	5%	-
Dr. A.I.M.M. Islam Consultant	5%	" 1000X12=12,000.00
Research Fellow (tobe appointed)	100%	" 3,000X12=36,000.00
Laboratory assistant	100%	" 2,000X12=24,000.00
Laboratory attendant	100%	" 1,500X12=18,000.00
	Sub-Total	Tk. 1,26,000.00

2. Supplies

Chemicals and Reagents, Immune reagents, antiglobulines, peroxidase canjugate, plastic and glass ware	Tk. 75,000.00
Sub-Total	Tk. 2,01,000.00

3. Equipment:

Microcentrifuge	Tk. 95,000.00
Electric incubator	Tk. 26,000.00
Travel and Transport	" 15,000.00
Transportation of things	" 5,000.00
Office Stationary	" 2,000.00
Printing and reproduction	" 3,000.00
Overhead contigencies	" 3,000.00
Sub-Total	Tk. 1,49,000.00
	Tk. 2,01,000.00
GRAND TOTAL Tk.	3,50,000.00
US$	9,210.52

Assignment of Investigators:

Dr. Muzahed Uddin Ahmed

-Collaborate with MMCH, IDH Mymensingh and BAU dairy farm for enrollment of subjects, collection of clinical information and biological samples.

- Ensure that careful processing, storage and cataloging of study sample is maintained.

Maintain an accruate record of all clinical epidemiological and laboratory information.

Processing samples i.e. characterization of Group A Rotavirus by Elisa and performance of PAGE.

Prof.S.Urasawa

- Provide monoclonal antibodies for serotyping, subgrouping of Group A Rotavirus strains.
- Provide facilities to perform hybridization of Rotavirus RNA with serotype specific oligonucleotide probes.
- Academic and strategic feedback.
- Antigenic characterization of typical and Atypical strains.

Dr.Nigar S. Shahid

Collaborate with Dr.M.U.Ahmed to assist in data analysis, interpretation and documentation.

Dr.M.A.Hamid Shaikh

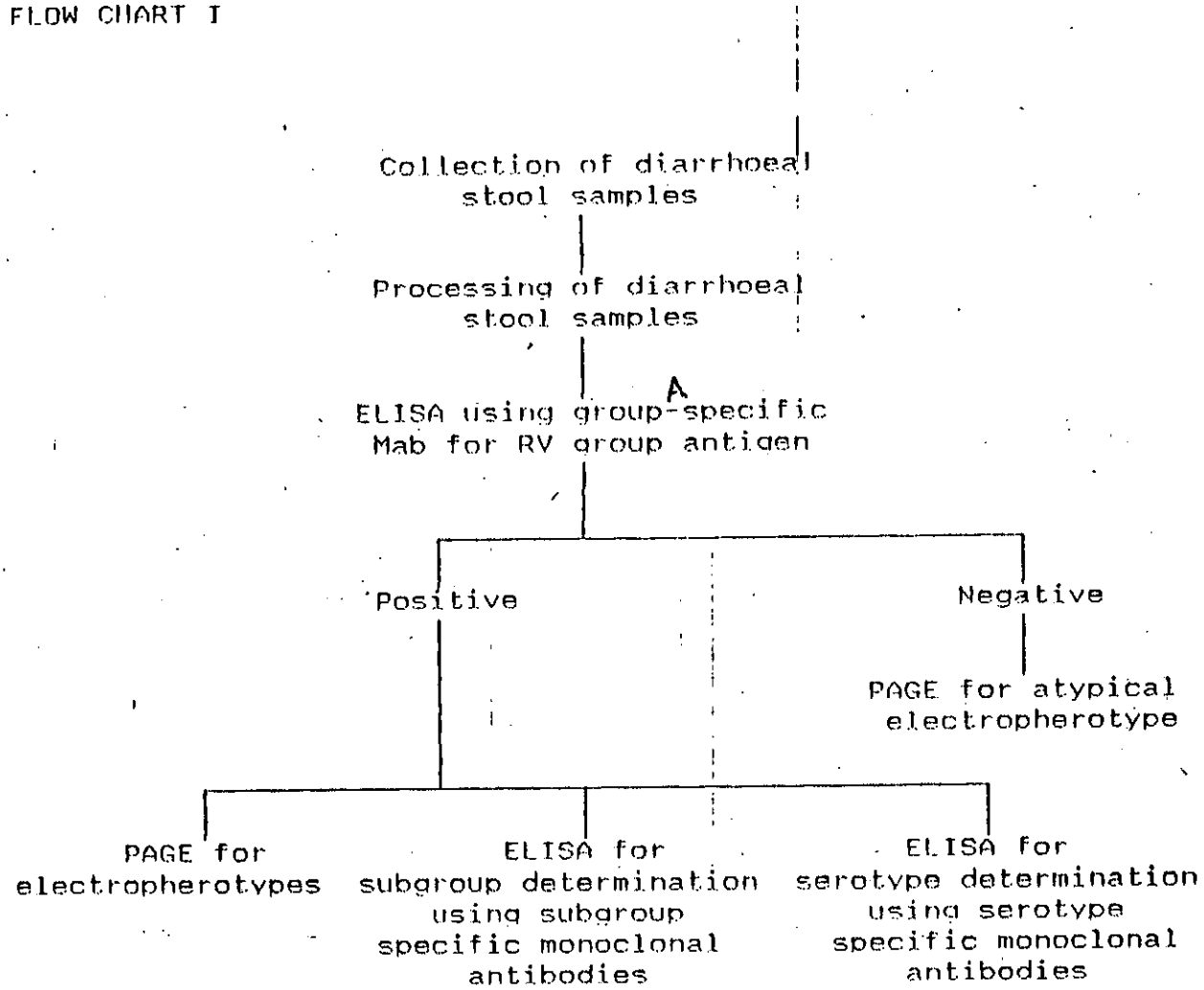
Collaborate with Dr.M.U.Ahmed to ensure enrollment of patients of the above hospitals.

- Record and maintain clinical information.

Prof. A.I.M.Mafakhkharul Islam

- Provide consultancy on matters relating to administration of this project and guide field staff to ensure smooth running of the protocol.

FLOW CHART I



NSS:mh/N3:BAU.FRM

CLINICAL RECORD SHEET OF DIARRHOEA PATIENTS (HUMAN)

Date of entry	|__|__|__|__|__|__|	1-6
Patient number	|__|__|__|	7-9
Sex	|__|	10
Age	|__|__|__|__| yy/mm	11-14
Date of diarrhoea onset	|__|__|__|__|__|__| yy/mm/dd	15-20
Date of hospitalization	|__|__|__|__|__|__|	21-26
Date of collection	|__|__|__|__|__|__|	27-32
Diarrhoea type	|__| 1=Loose; 2=Watery; 3=Watery mucoid	33
No. of stool in last 24 hours	|__|__|	34-35
Vomiting	|__| 0=No; 1=Yes	36
No. of vomit in last 24 hours	|__|__|	37-38
Thirst in last 24 hours	|__| 1=Usual; 2=<Usual; 3=>Usual; 4=Not severe	39
Urine output in last 24 hours	|__|	40
Fever	|__| 0=No; 1=Yes	41
Fever (duration in days)	|__|__|	42-43
Temperature	|__|__|__|__| ^{o}C	44-47
Pulse character	|__| 1=Normal; 2=Rapid; 3=Rapid/Feeble/Unpalpable	48
Pulse rate/min	|__|__|__|	49-51
Abdominal pain	|__| 0=No; 1=Yes	52
Dehydration status	|__| 1=Normal; 2=Mild; 3=Moderate 4=Severe	53

CLINICAL DIAGNOSIS - In case of children <2 years of age

Is child usually breast-fed	|__| 0=No; 1=Yes	54
Was child breast-fed during diarrhoea episode	|__| 0=No; 1=Yes	55

NSS:mh/N3:BAU.FRM

CLINICAL RECORD SHEET OF DIARRHOEA IN CALF

Date of entry	|__|__|__|__|__|__|	1-6
Calf number	|__|__|__|	7-9
Age	|__|__|__|__| yy/mm	10-13
Species	|__|	14
Breed	|__|	15
Sex	|__|	16
Location	|__|__|__|__|__|__|__|__|	17-24
Date of diarrhea onset	|__|__|__|__|__|__| yy/mm/dd	25-30
Date of collection	|__|__|__|__|__|__|	31-36
Diarrhoea type	|__| 1=Loose: 2=Watery: 3=Watery mucoid	37

CLINICAL DIAGNOSIS - In case of calf <1 year of age

Is calf usually colostrum fed	|__|	0=No: 1=Yes	38
Was calf colostrum/milk fed during diarrhoea episode	|__|	0=No: 1=Yes	39

NSS:mh/N3:BAU.FRM

Abstract Summary (ERC)

1. Patients with watery diarrhoea belonging to all age groups will be enrolled for the study.

2. There will be no potential risk to the subjects.

3. NA

4. All information will be coded, Raw data will be kept in locked cabinets.

5. Signed informed consent will be obtained from authorized legal guardians or parent of the minor subjects and the patient himself/herself from adults.

6. Five to ten minutes will be required for the interview which will be conducted at bedside.

7. There is no potential risk involved with this investigation. The benifit is that since rotavirus tests are only performed at ICDDR,B, this is a unique opportunity to set up similar tests at a district level which is very much required for the appropriate treatment of acute watery diarrhoea.

8. Diarrhoeal stool alone is required for the investigation.

Consent Form

Rotavirus (RV) is a common problem in young children in Bangladesh. Mortality due to rotavirus disease is high. The treatment of RV is based on rehydration with oral rehydration solution (ORS) and other common fluids. Diagnosis of this disease is important to dissuade use of antibiotics in childhood diarrhoea.

The identification of RV is performed by a simply technique which is not present in Mymensingh. If this technique is established here this will provide guideline to doctors for prompt and early treatment of RV diarrhoea.

We shall appreciate your/your ward joining the study and require a small amount of stool sample from the patient. We shall talk to you for five to ten minutes regarding the clinical condition.

If you/your ward decides to participate, please put your signature thumb impression on the following place.

Signature of guardian/self.....

Signature of investigator .....

N2/ERC:NS

সম্মতিপত্র

বাংলাদেশে ছোট শিশুদের মধ্যে রোটাভাইরাস রোগ একটি সাধারণ সমস্যা। এই রোগে মৃত্যুর হার অধিক। রোটাভাইরাস চিকিৎসার ভিত্তি হলো মুখে খাবার স্যালাইন ও অন্যান্য তরল জাতীয় খাবারের মাধ্যমে পানি স্বল্পতা দূর করন। শিশুদের পাতলা পায়খানায় এন্টিবায়োটিক অপব্যবহারের ক্ষেত্রে এই রোগ নির্ণয় অতি জরুরী।

রোটাভাইরাস রোগ নির্ণয়ের সহজ পদ্ধতি সম্প্রতি বের হয়েছে। যদি এখানে এই পদ্ধতি চালু করা যায় তাহলে ডাক্তারদের দ্রুত এবং আগে ভাগে রোটাভাইরাস ডায়রিয়া চিকিৎসার দিক নির্দেশনায় সহায়ক হবে।

আমরা আপনার এবং আপনার সন্তানাদির এই গবেষণা প্রকল্পে অংশ গ্রহনের জন্য ধন্যবাদ জানাচ্ছি এবং রোগীর কাছ থেকে স্বল্প পরিমান পায়খানার নমুনার গোপনীয়তা রক্ষা করছি। আমরা আপনার সঙ্গে পাঠ কা[illegible] দিয়া নির্দিষ্ট রোগীর অবস্থা সম্পর্কে আলোচনা করিব।

আপনি বা আপনার সন্তানাদি যদি আমাদের গবেষণায় অংশ গ্রহন করিতে চান তাহলে নীচে দস্তখত অথবা টিপসই করার জন্য অনুরোধ করছি।

অভিভাবকের / নিজের স্বাক্ষর - - - - - - - - -

অনুসন্ধানকারীর স্বাক্ষর - - - - - - - - - - -

তারিখ - - - - - -